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The *FTO*, *PNPLA3* and *TM6SF2* Gene Polymorphisms and Genetic Predisposition to NAFLD in Yakut Population

Aleksandra T. Diakonova*; Khariton A. Kurtanov, PhD; Nadezhda I. Pavlova, PhD; Tuyara N. Aleksandrova

Yakut Science Center of Complex Medical Problems
Yakutsk, the Republic of Sakha (Yakutia), Russia

Abstract

The aim of our research was to study the distribution of alleles and genotypes of the *FTO* rs9939609 SNP, the *PNPLA3* rs738409 SNP, and the *TM6SF2* rs58542926 SNP in the Yakut population.

Methods and Results: A total of 85 DNA samples from the population were tested. An analysis of the frequency distribution of alleles and genotypes of the *FTO* rs9939609 SNP in the study group did not reveal significant differences. An analysis of the frequency distribution of alleles and genotypes of the *PNPLA3* rs738409 SNP revealed that in men and women the G allele and the homozygous GG genotype prevailed. The results of the analysis of the frequency distribution of alleles and genotypes of the *TM6SF2* rs58542926 SNP showed the predominance of individuals with the C allele (89% in men and 90% in women) with statistical significance in women.

Conclusion: The further studies with a larger sample size are required to detect the features of the distribution of alleles and genotypes of the *FTO* rs9939609 SNP and the *TM6SF2* rs58542926 SNP in that population. (**International Journal of Biomedicine. 2021;11(1):92-95.**)

Key Words: nonalcoholic fatty liver disease • single nucleotide polymorphism • genome-wide association study • Yakuts

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Abbreviations

bp, base pairs; **NAFLD**, nonalcoholic fatty liver disease; **PNPLA3**, patatin-like phospholipase domain-containing protein 3; **SNP**, single nucleotide polymorphism; **TM6SF2**, transmembrane 6 superfamily member 2

Introduction

A person's genetic predisposition determines the risk of developing NAFLD. However, not all at-risk individuals develop the disease, suggesting that most complex multifactorial diseases are the result of interactions between genes and the environment. The onset or severity of the disease may differ in individuals with the same genotype in different environmental conditions, or vice versa, confirming that the phenotype is a consequence of genotype-environmental interactions; diet, lifestyle, exposure to chemicals and toxins constitute the bulk of environmental risks. Most diseases of the modern lifestyle, such as diabetes, cardiovascular diseases, hypertension, and obesity, are usually transmitted by a multifactorial mode of inheritance. This term refers to a complex type of inheritance, which involves a combination of both genetic and other factors, including the environment.[1] Nonalcoholic fatty liver disease (NAFLD) is one of the most important complex and multifactorial lifestyle diseases. NAFLD initiates from extra fat storage in the liver and can advance to hepatitis, fibrosis, liver failure, and hepatocellular carcinoma. NAFLD is often associated with obesity, diabetes, and hyperlipidemia. The prevalence of the disease varies markedly in different populations. It ranges from 20% to 30% in Western countries,[2] from 20% to 30% in Europeans,[3] 8% to 9% in Japan,[4] and 25%-30% in India.[5] The overall

***Corresponding author:** Aleksandra T. Diakonova. Yakut Science Center of Complex Medical Problems. Yakutsk, the Republic of Sakha (Yakutia), Russia. E-mail: dyakonovaa@bk.ru*

prevalence of NAFLD in Asia has so far been estimated at 29.6%. Due to significant changes in lifestyle and dietary habits, NAFLD has become a major social health burden in China, with a prevalence of 18.2% in 2000-2006, 20.0% in 2007-2009, and 20.9% in 2010–2013. A recent meta-analysis found that the national prevalence of NAFLD in China reached 29.1%.[6]

Understanding genetic predisposition has been a major focus of recent research, in addition to changes in dietary habits and lifestyle modifications that have been shown to benefit patients with NAFLD and help better control the disease. [1] The GWAS identified several genes as a major genetic determinant for the predisposition to NAFLD. The association between SNPs in the fat mass and obesity-associated (*FTO*) gene and BMI and obesity has been confirmed in multiple populations.[7-13] Gerken et al.[14] showed that FTO shares sequence motifs with Fe(II)- and 2-oxoglutarate–dependent oxygenates, which allows it to alter DNA methylation and regulate gene transcription. The *FTO* gene encodes one of the lipolysis regulators, which is involved in the control of adipocyte differentiation, energy homeostasis, and leptin-independent appetite control. The FTO SNPs associated with adiposity are intronic and may exert functional effects through altered expression of FTO mRNA. According to the results of previous studies, the A allele of the *FTO* rs9939609 is associated with decreased lipolysis, impaired appetite control, and a lack of satiety after an adequate meal.[15-18] The phenotypic manifestation of the A allele of the *FTO* gene is overweight and obesity due to overeating,[18,19] which in turn is one of the most common risk factors for the development of NAFLD.

Molecular genetic studies have shown that the *PNPLA3* gene, located on the long arm of chromosome 22q13.31, is expressed in the membranes of hepatocytes and is responsible for intrahepatic lipid metabolism by coding for the synthesis of adiponutrin, a protein that regulates the activity of triacylglycerolipase in adipocytes. The most significant polymorphism in the *PNPLA3* gene is I148M (rs738409). The most prominent variant is the *PNPLA3* rs738409 [G], which is a nonsynonymous substitution of cytosine for guanine (C>G) that changes codon 148 from encoding isoleucine (I) to methionine (M) (I>M, I148M).[20,21] Wild-type (148I) *PNPLA3* has lipolytic activity towards triglycerides.[22,23] The 148M mutation leads to the development of macrovescicular steatosis.[22,23] In humans, the *PNPLA3* I148M mutation has been shown to influence not only intrahepatic remodeling but also reduces very low density lipoproteins (VLDL) secretion.[24] Recently, a study showed that carriers of the rs738409[G] allele have lower de novo lipogenesis as compared to non-carriers due to a reduction in liver SREBP1c mRNA levels.[25] However, the issue of the functional consequences of the I148M polymorphism is therefore still intensively debated.[26]

In 2014, the significance of rs58542926 in the transmembrane 6 superfamily member 2 (*TM6SF2*) gene in NAFLD was found for the first time.[27] The *TM6SF2* rs58542926 is a substitution of guanine by adenine in nucleotide 499, which leads to the replacement of glutamic acid by lysine in amino acid residue 167(E167K).[27] Recent studies have found that *TM6SF2* rs58542926 was a significant risk factor for the development of NAFLD.[28,29]

The aim of our research was to study the distribution of alleles and genotypes of the *FTO* rs9939609 SNP, the *PNPLA3* rs738409 SNP, and the *TM6SF2* rs58542926 SNP in the Yakut population.

Materials and Methods

The study of the *FTO* rs9939609, SNP the *PNPLA3* rs738409 SNP, and the *TM6SF2* rs58542926 SNP was carried out in the Department of Molecular Genetics at YSC CMP. For the study, we used DNA samples from the collection of biomaterials of the YSC KP using the UNU "Genome of Yakutia" (reg. No. USU_507512). A total of 85 DNA samples from the population were tested.

The inclusion criteria for the study were Yakuts by ethnicity, living in Yakutia, without liver damage by chronic viral hepatitis. Exclusion criteria: autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis, hereditary hemochromatosis, Wilson-Konovalov disease, and alcohol abuse (>30g/l).

Genomic DNA samples were isolated from peripheral blood lymphocytes using a commercial DNA kit, Excel Biotech (Yakutsk). The study SNPs were analyzed by PCR-RFLP reaction. The gene regions containing the polymorphic variants were amplified with standard pairs of primers produced by Biotech-Industry LLC (Moscow, Russia). The reaction mixture (25 μl) for PCR contained of forward and reverse primer (10 pmol/μl) (1μl) (Moscow, Russia), Dream Taq PCR master mix (12.5 μl), deionized water (9.5 μl), and DNA in the amount of 100μg/ml (1μl). A mixture for RFLP (20μL) consisted of amplificate (7μL), deionized water (10.9μL), restriction buffer (2μL), and restriction endunuclease Hpy188I (2 u.a.) for the *TM6SF2* gene, Zrm I (2 u.a.) for the gene *FTO,* and BstF5VI (2 e.a.) for the *PNPLA3* gene.

The temperature-time regime for PCR is optimized for amplification of this nucleotide sequence and is presented in Table 1.

Table 1.

Conditions for PCR

Gene	Amplification	The length of the restriction fragments	PCR conditions
TM6SF2	429 bp	CC–178 bp, 166 bp, 85 bp TT–251, 178 bp CT–251 bp, 178 bp, 166 bp, 85 bp	1. 94 °C – 10 min 2. (94 °C – 1 min; 62 °C – 40 sec; 72 °C – 1 min) – 40 cycles 3. 72 °C – 10 min
FTO	182 bp	AA–154 bp, 28 bp AT–154 bp, 28 bp, 182 bp TT–182 bp	1. 95 °C – 4 min 2. (94 °C – 30 sec; 58 °C – 30 sec; 72 °C – 1 min) – 35 cycles 3. 72 °C – 10 min
PNPLA3	333 bp	CC–200 bp, 133 bp GC–333 bp, 200 bp, 133 bp GG– 333 bp	1. 95 °C – 5 min 2. (94 °C – 30 sec; 66 °C – 30 sec; 72 °C – 40 sec) – 37 cycles 3. 72 °C – 5 min

PCR products were detected by horizontal electrophoresis in a 2% agarose gel plate with the addition of ethidium bromide, a specific intercalating fluorescent DNA (RNA) dye, using a standard Tris-acetate buffer at a field voltage of ~ 20V/cm for 30 minutes. RFLP products were detected by horizontal electrophoresis in 4% agarose gel stained with ethidium bromide using a standard Tris-acetate buffer at 120V for 1 hour.

Statistical analysis was performed using the Statistica 8.0 software package (Stat-Soft Inc., USA). The correspondence of the distributions of genotypes to the expected values at HWE and comparison of the frequencies of allelic variants/genotypes were performed using the chi-square test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

An analysis of the frequency distribution of alleles and genotypes of the FTO rs9939609 SNP in the study group did not reveal significant differences (Table 2). An analysis of the frequency distribution of alleles and genotypes of the PNPLA3 rs738409 SNP revealed that in men and women the G allele and the homozygous GG genotype prevailed (Table 3). The results of the analysis of the frequency distribution of alleles and genotypes of the TM6SF2 rs58542926 SNP showed the predominance of individuals with the C allele (89% in men and 90% in women) with statistical significance in women (Table 4).

Table 2.

Frequency distribution of alleles and genotypes of the FTO rs9939609 SNP in the study group

FTO	n		Genotype, %			Allele, %		χ^2	*P*
			AA	AT	TT	A	T		
All	85	O	4	30	66	19	81	0.012	0.912
		E	4	30,5	65,5				
Woman	63	E	13	27	60	26	74	5.748	0.0165
		E	7	39	54				
Man	22	O	4	32	64	20	80	0.011	0.917
		E	4	32	64				

Note: O - observed; E - expected.

Table 3.

Frequency distribution of alleles and genotypes of the PNPLA3 rs738409 SNP in the study group

PNPLA3	n		Genotype, %			Allele, %		χ^2	*P*
			CC	CG	GG	C	G		
All	85	O	14	33	53	31	69	4.274	0.0387
		E	9	43	48				
Woman	63	O	13	32	55	29	71	3.11	0.0778
		E	8	41	51				
Man	22	O	18	36	45	36	64	1.01	0.3149
		E	13	46	40				

The frequency of occurrence of the mutant alleles of the study SNPs in various populations, according to Ensembl.org, is presented in Table 5.

Table 4.

Frequency distribution of alleles and genotypes of the TM6SF2 rs58542926 SNP in the study group

TM6SF2	n		Genotype, %			Allele, %		χ^2	*P*
			CC	CT	TT	C	T		
All	85	O	84	12	4	89	11	12.187	0.0005
		E	80	19	1				
Woman	63	O	84	11	5	90	10	10.059	0.0015
		E	80	18	1				
Man	22	O	82	14	4	89	11	2.296	0.1297
		E	79	20	1				

Table 5.

The frequency of occurrence of the mutant alleles (%) of the study SNPs in various populations, according to Ensembl.org

SNP	Mutant allele	Yakuts	Puerto Ricans	Peruvians	Finns	South Han Chinese
rs9939609 *FTO*	A	19	36	8	39	14
rs738409 *PNPLA3*	G	69	32	72	17	38
rs58542926 *TM6SF2*	T	11	9	5	6	11

We previously found a high frequency of the *PNPLA3* (rs738409) [G] allele in the Yakut population (72%-73%).[30,31] The accumulation of triglycerides in hepatocytes, associated with the *PNPLA3* p.I148M variant, was probably an adaptation to a cold climate; this accumulation is not needed in the modern world, but leads to NAFLD among the Yakut population. As noted by many domestic and foreign researchers, carriers of the *PNPLA3* G allele are more susceptible to liver diseases (NAFLD, NASH) with a high risk of developing cirrhosis and hepatocellular carcinoma.[20]

Undoubtedly, further studies with a larger sample size are required to detect the features of the distribution of alleles and genotypes of the FTO rs9939609 SNP and the TM6SF2 rs58542926 SNP in that population.

Competing Interests

The authors declare that they have no competing interests.

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